**Program/Abstract # 295**
**Cdx1 is a required activator of *Hox* expression during gastrulation**
Abraham Fainsod [1], Rachel Ben-Haroush Schyr [1], Kristen Correia [2], Yehuda Shabtai [1], Cooduvalli S. Shashikant [3], Robb Krumlauf [2]
[1] *Dept. of Cell. Biochem. and Hum. Genet., Hebrew Univ., Jerusalem, Israel*
[2] *Stowers Institute for Medical Research, Kansas City, MO, USA*
[3] *Dept. of Dairy and Animal Sci., College of Agri. Sci., Pennsylvania State Univ., PA, USA*

Early patterning of the anterior–posterior axis is achieved primarily by a family of homeobox genes, the *Hox* genes. The *Hox* genes are organized in clusters, and the gene order along the cluster is the same as their order of expression along the anterior–posterior axis, and the sequence of their activation during early embryogenesis. This connection between cluster organization and temporal and spatial expression has been termed co-linearity. The regulation of co-linearity has been the focus of intensive research. The *caudal*-type genes, the *Cdx* genes, are candidate regulators of *Hox* co-linearity. The *Cdx* genes are activated just prior to the onset of gastrulation taking a caudal position as the embryo elongates. Changes in *Cdx* activity result in changes in *Hox* expression patterns. Manipulation of Cdx1 levels changes the timing of *HoxC8* activation, demonstrating that Cdx1 activity is required for the timely initiation of *HoxC8* expression. Through mutation analysis we identified three pairs of Cdx binding sites. We show that, these sites are bound *in vivo* by the Cdx1 protein at the time *HoxC8* is activated in the embryo. Analysis of *HoxB1* activation during gastrulation also showed a *Cdx1* requirement, which translates into an abnormal timing of activation as a result of Cdx1 manipulation. These results place the *Cdx* genes as early activators of *Hox* expression, possibly involved in the establishment of *Hox* temporal co-linearity.

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**Program/Abstract # 296**
**Role of REEP4 in early *Xenopus* development**
Joanna Argasinska, Alice Young, Amer Rana, Jim C. Smith
*Wellcome Trust/CR UK Gurdon Institute and Dept. of Zoology, University of Cambridge, Cambridge, UK*

REEP (receptor expression enhancing protein) 4 was identified in the course of a functional antisense morpholino oligonucleotide (MO) screen searching for genes involved in the early development of *Xenopus tropicalis*. Knockdown of this protein in both *X. tropicalis* (using two non-overlapping MOs) and *X. laevis* causes embryos to develop a kinked body axis and to be paralyzed. REEPs are highly conserved among vertebrates and contain a TB2/DP1, HVA22 domain involved in intracellular trafficking and secretion. Consistent with this, REEPs1 and 3 have been shown to function in intracellular trafficking, enhancing the expression of odorant and taste receptors in mammals. At the tailbud stage of *Xenopus* development, REEP4 is strongly expressed in somites and weakly in the neural tube, although at later stages neural staining becomes stronger. Overexpression of a GFP-tagged form of the protein localizes to the cytoplasmic membrane, but neither this construct nor untagged versions of REEP4 cause any defects in development. Paralysis in embryos lacking REEP4 might be caused by defects in the nervous system or in muscle development. We have examined the expression of Islet1/2 (to mark motor neurons) and 12/101 (to label muscle) at tailbud stages and found that while expression levels of Islet1/2 appear relatively normal, muscle development is impaired. However, there is a reduction in Islet1/2 expression at later stages. We are currently investigating the effect of losing REEP4 on muscle gene expression, asking whether its loss affects predominantly slow or fast muscle and investigating its mode of action.

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**Program/Abstract # 297**
**New roles for voltage gated calcium channel beta subunits in zebrafish**
Alicia M. Ebert [1], Catherine A. McAnelly [1], Anne Handschy [1], Kathryn E. Gately [1], William A. Horne [2], Deborah M. Garrity [1]
[1] *Dept. of Biology, Colorado State University, Fort Collins, CO, USA*
[2] *College of Veterinary Medicine, Cornell University, Ithaca, NY, USA*

Voltage gated calcium channels are heteromeric complexes comprised of a pore forming alpha1 subunit and several auxiliary subunits. The beta subunits (beta1–beta4) chaperone the alpha1 subunit to the membrane and modulate the gating properties of the calcium channel. Mutations in the beta4 subunit lead to ataxia, lethargic behavior and seizures in mice and have been associated with epilepsy in humans. Since known mutant alleles of beta4 are not embryonic lethal, the functions of the beta4 protein in development are unclear. Zebrafish have two beta4 subunits which encode 5-domain proteins similar in structure to the human beta4 subunit. These genes are expressed ubiquitously before gastrulation but later become restricted to distinct regions in the developing central nervous system and heart. Targeted knockdown of beta4 genes by morpholino leads to arrest or delay of epiboly, accompanied by cell death along the blastoderm margin and embryo death. This unusual phenotype can be rescued by injection of wild-type beta4 RNA into the yolk syncytial layer (YSL). The YSL nuclei in beta4 morphants have abnormal size and distribution, consistent with defects in mitosis and nuclear attachment to the cytoskeleton. Injection of excess beta4 RNA leads to gain-of-function phenotypes that also perturb epiboly. Since a requirement for calcium channel function has not